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IL-8-like Proteins

This invention relates to the novel proteins, termed INSP093 and INSP094, herein identified as secreted proteins, in particular, as members of the Interleukin (IL) 8-like chemokine family and to the use of these proteins and nucleic acid sequences from the
5 encoding genes in the diagnosis, prevention and treatment of disease.

All publications, patents and patent applications cited herein are incorporated in full by reference.

Background

The process of drug discovery is presently undergoing a fundamental revolution as the era
10 of functional genomics comes of age. The term "functional genomics" applies to an approach utilising bioinformatics tools to ascribe function to protein sequences of interest. Such tools are becoming increasingly necessary as the speed of generation of sequence data is rapidly outpacing the ability of research laboratories to assign functions to these protein sequences.

15 As bioinformatics tools increase in potency and in accuracy, these tools are rapidly replacing the conventional techniques of biochemical characterisation. Indeed, the advanced bioinformatics tools used in identifying the present invention are now capable of outputting results in which a high degree of confidence can be placed.

Various institutions and commercial organisations are examining sequence data as they
20 become available and significant discoveries are being made on an on-going basis. However, there remains a continuing need to identify and characterise further genes and the polypeptides that they encode, as targets for research and for drug discovery.

Introduction**Secreted Proteins**

25 The ability for cells to make and secrete extracellular proteins is central to many biological processes. Enzymes, growth factors, extracellular matrix proteins and signalling molecules are all secreted by cells. This is through fusion of a secretory vesicle with the plasma membrane. In most cases, but not all, proteins are directed to the endoplasmic reticulum and into secretory vesicles by a signal peptide. Signal peptides are cis-acting sequences
30 that affect the transport of polypeptide chains from the cytoplasm to a membrane bound

compartment such as a secretory vesicle. Polypeptides that are targeted to the secretory vesicles are either secreted into the extracellular matrix or are retained in the plasma membrane. The polypeptides that are retained in the plasma membrane will have one or more transmembrane domains. Examples of secreted proteins that play a central role in the functioning of a cell are cytokines, hormones, extracellular matrix proteins (adhesion molecules), proteases, and growth and differentiation factors. Description of some of the properties of these proteins follows.

Chemokines

These signalling molecules are distinct from cytokines and are responsible for inducing chemotaxis or directed migration. They are highly specific, a fact which is illustrated by the fact that IL-8 is chemotactic to granulocytes but not monocytes. Chemokines contain four conserved cysteine residues and are divided into three families, α (CXC), β (CC) and γ (C), based on the position of conserved cysteine residues. If the first two cysteines are separated by another amino acid, then the chemokine is a member of the α family, while the first two cysteine residues are next to each other in the β family members. Members of the γ family only have one cysteine residue, rather than two, in their N-terminus. In the α and β families, disulphide bonds are formed between the first and third and the second and fourth residues.

Specificity of chemokines depends on the presence of specific receptors on cell surfaces. Chemokines have been shown to play a role in the migration of leukocytes. Upon activation, remodeling of the cytoskeleton of leukocytes is induced allowing the cell to flatten and pass from an intravascular space into a tissue space. Interaction of chemokines with seven-transmembrane G-protein coupled receptors leads to rapid accumulation of intracellular free calcium in the responding cells. This mobilisation is critical for chemotaxis, respiratory burst and upregulation of adhesive interactions of leukocytes. Chemokines have also been shown to regulate the expression of adhesion molecules on neutrophils, monocytes, lymphocytes and eosinophils. For example, MIP-1 α and RANTES cause adhesion of monocytes to endothelium while MIP-1 β induces CD8⁺ T-cell adhesion to endothelium.

Increasing knowledge of these domains is therefore of extreme importance in increasing the understanding of the underlying pathways that lead to the disease states and associated

disease states mentioned above, and in developing more effective gene and/or drug therapies to treat these disorders.

THE INVENTION

The invention is based on the discovery that the INSP093 and INSP094 polypeptides are
5 IL-8 like chemokines.

In one embodiment of the first aspect of the invention, there is provided a polypeptide which:

- (i) comprises the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4 and/or SEQ ID NO:6;
- 10 (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine family, or has an antigenic determinant in common with the polypeptides of (i); or
- (iii) is a functional equivalent of (i) or (ii).

Preferably, the polypeptide according to this first aspect of the invention:

- (i) comprises the amino acid sequence as recited in SEQ ID NO:6;
- 15 (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine family, or has an antigenic determinant in common with the polypeptides of (i); or
- (iii) is a functional equivalent of (i) or (ii).

According to a second embodiment of this first aspect of the invention, there is provided a polypeptide which:

- 20 (i) consists of the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4 and/or SEQ ID NO:6;
- (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine family, or having an antigenic determinant in common with the polypeptides of (i); or
- 25 (iii) is a functional equivalent of (i) or (ii).

The polypeptide having the sequence recited in SEQ ID NO:2 is referred to hereafter as "INSP093 exon A polypeptide". The polypeptide having the sequence recited in SEQ ID NO:4 is referred to hereafter as "INSP093 exon B polypeptide". The polypeptide having the sequence recited in SEQ ID NO:6 is referred to hereafter as the "INSP093 partial

polypeptide”.

In consideration of the fact that there is no methionine start codon at the beginning of the INSP093 exon A nucleotide sequence (SEQ ID NO:1), and it is considered very likely that there may be further exons at the 5' end of SEQ ID NO:1 that will provide amino acids
5 that are N-terminal to the beginning of the sequence given in SEQ ID NO:2. It is also noticeable that there is no stop codon at the 3' end of INSP093 exon B (SEQ ID NO:3), thus it is also considered very likely by the Applicant that there are further exons 3' to SEQ ID NO:3 in the genome that will provide amino acids that are C-terminal to the end of the sequence given in SEQ ID NO:4.

- 10 The term “INSP093 polypeptides” as used herein includes polypeptides comprising the INSP093 exon A polypeptide, the INSP093 exon B polypeptide and the INSP093 partial polypeptide.

In a third embodiment of the first aspect of the invention, there is provided a polypeptide which:

- 15 (i) comprises the amino acid sequence as recited in SEQ ID NO:8, SEQ ID NO:10 and/or SEQ ID NO:12;
- (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine family, or has an antigenic determinant in common with the polypeptides of (i); or
- (iii) is a functional equivalent of (i) or (ii).

- 20 Preferably, the polypeptide according to this first aspect of the invention:

- (i) comprises the amino acid sequence as recited in SEQ ID NO:10;
- (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine family, or has an antigenic determinant in common with the polypeptides of (i); or
- (iii) is a functional equivalent of (i) or (ii).

- 25 According to a fourth embodiment of this first aspect of the invention, there is provided a polypeptide which:

- (i) consists of the amino acid sequence as recited in SEQ ID NO:8, SEQ ID NO:10 and/or SEQ ID NO:12;
- (ii) is a fragment thereof which functions as a member of the IL-8 like chemokine

family, or having an antigenic determinant in common with the polypeptides of (i);
or

(iii) is a functional equivalent of (i) or (ii).

The polypeptide having the sequence recited in SEQ ID NO:8 is referred to hereafter as
5 "INSP094 exon A polypeptide". The polypeptide having the sequence recited in SEQ ID
NO:10 is referred to hereafter as the "INSP094 partial polypeptide".

In consideration of the fact that there is no methionine start codon at the beginning of the
INSP094 exon A nucleotide sequence (SEQ ID NO:7), and it is considered very likely that
there may be further exons at the 5' end of SEQ ID NO:7 that will provide amino acids
10 that are N-terminal to the beginning of the sequence given in SEQ ID NO:2. It is also
noticeable that there is no stop codon at the 3' end of INSP094 exon A (SEQ ID NO:7),
thus it is also considered very likely by the Applicant that there are further exons 3' to SEQ
ID NO:7 in the genome that will provide amino acids that are C-terminal to the end of the
sequence given in SEQ ID NO:8.

15 The term "INSP094 polypeptides" as used herein includes polypeptides comprising the
INSP094 exon A polypeptide and the INSP094 partial polypeptide.

By "functions as a member of the IL-8 like chemokine family" we refer to polypeptides
that comprise amino acid sequence or structural features that can be identified as conserved
features within the polypeptides of the IL-8 like chemokine family, such that the
20 polypeptide's interaction with ligand is not substantially affected detrimentally in
comparison to the function of the full length wild type polypeptide. In particular, we refer
to the presence of cysteine residues in specific positions within the polypeptide that allow
the formation of intra-domain disulphide bonds. Ability to function as an IL-8 like
chemokine may be measured using an assay kit such as the Human IL-8 ELISA (IBL,
25 Hamburg) which can detect IL-8 concentrations as low as 70pg/ml.

Studies on structure-activity relationships indicate that chemokines bind and activate
receptors by making use of the amino-terminal region. Proteolytic digestion, mutagenesis,
or chemical modifications directed to amino acids in this region can generate compounds
having antagonistic activity (Loetscher P and Clark-Lewis I, J Leukoc Biol, 69: 881-884,
30 2001 Lambeir A, *et al.* J Biol Chem, 276: 29839-29845, 2001, Proost P, *et al.* Blood, 98
(13):3554-3561, 2001). Thus, antagonistic molecules resulting from specific modifications

(deletions, non-conservative substitutions) of one or more residues in the amino-terminal region or in other regions of the corresponding chemokine are considered to have therapeutic potential for inflammatory and autoimmune diseases (WO 02/28419; WO 00/27880; WO 99/33989; Schwarz MK and Wells TN, Curr Opin Chem Biol, 3: 407-17, 5 1999). Therefore, a further object of the present patent application is represented by such kind of antagonists generated by modifying the polypeptides of the invention.

The therapeutic applications of the polypeptides of the invention and of the related reagents can be evaluated (in terms of safety, pharmacokinetics and efficacy) by the means of the *in vivo* / *in vitro* assays making use of animal cell, tissues and models (Coleman RA 10 *et al.*, Drug Discov Today, 6: 1116-1126, 2001; Li AP, Drug Discov Today, 6: 357-366, 2001; Methods Mol. Biol vol. 138, "Chemokines Protocols", edited by Proudfoot AI *et al.*, Humana Press Inc., 2000; Methods Enzymol, vol. 287 and 288, Academic Press, 1997), or by the means of *in silico* / computational approaches (Johnson DE and Wolfgang GH, Drug Discov Today, 5: 445-454, 2000), known for the validation of chemokines and other 15 biological products during drug discovery and preclinical development.

The present application discloses novel chemokine-like polypeptides and a series of related reagents that may be useful, as active ingredients in pharmaceutical compositions appropriately formulated, in the treatment or prevention of diseases such as cell proliferative disorders, autoimmune/inflammatory disorders, cardiovascular disorders, 20 neurological disorders, developmental disorders, metabolic disorder, infections and other pathological conditions. In particular, given the known properties of chemokines, the disclosed polypeptides and reagents should address conditions involving abnormal or defective cell migration. Non-limitative examples of such conditions are the following: arthritis, rheumatoid arthritis (RA), psoriatic arthritis, osteoarthritis, systemic lupus 25 erythematosus (SLE), systemic sclerosis, scleroderma, polymyositis, glomerulonephritis, fibrosis, lung fibrosis and inflammation, allergic or hypersensitivity diseases, dermatitis, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, multiple sclerosis, septic shock, HIV infection, transplant rejection, wound healing, metastasis, endometriosis, hepatitis, liver fibrosis, 30 cancer, analgesia, and vascular inflammation related to atherosclerosis.

In a second aspect, the invention provides a purified nucleic acid molecule which encodes a polypeptide of the first aspect of the invention.

Preferably, the purified nucleic acid molecule comprises the nucleic acid sequence as recited in SEQ ID NO:1 (encoding the INSP093 exon A polypeptide), SEQ ID NO:3 (encoding the INSP093 exon B polypeptide), SEQ ID NO:5 (encoding the INSP093 partial polypeptide), SEQ ID NO:7 (encoding the INSP094 exon A polypeptide) and/or SEQ ID NO:9 (encoding the INSP094 partial polypeptide) or is a redundant equivalent or fragment of any one of these sequences.

The invention further provides that the purified nucleic acid molecule consists of the nucleic acid sequence as recited in SEQ ID NO:1 (encoding the INSP093 exon A polypeptide), SEQ ID NO:3 (encoding the INSP093 exon B polypeptide), SEQ ID NO:5 (encoding the INSP093 partial polypeptide), SEQ ID NO:7 (encoding the INSP094 exon A polypeptide) and/or SEQ ID NO:9 (encoding the INSP094 partial polypeptide) or is a redundant equivalent or fragment of any one of these sequences.

In a third aspect, the invention provides a purified nucleic acid molecule which hybridizes under high stringency conditions with a nucleic acid molecule of the second aspect of the invention.

In a fourth aspect, the invention provides a vector, such as an expression vector, that contains a nucleic acid molecule of the second or third aspect of the invention.

In a fifth aspect, the invention provides a host cell transformed with a vector of the fourth aspect of the invention.

In a sixth aspect, the invention provides a ligand which binds specifically to protein members of the IL-8 like chemokine family of the first aspect of the invention.

In a seventh aspect, the invention provides a compound that is effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

A compound of the seventh aspect of the invention may either increase (agonise) or decrease (antagonise) the level of expression of the gene or the activity of the polypeptide.

Importantly, the identification of the functions of the INSP093 and INSP094 polypeptides allows for the design of screening methods capable of identifying compounds that are effective in the treatment and/or diagnosis of disease. Ligands and compounds according to the sixth and seventh aspects of the invention may be identified using such methods. These methods are included as aspects of the present invention.

In an eighth aspect, the invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in therapy or diagnosis of diseases in which members of the IL-8 like chemokine family are implicated. Such diseases may include cell proliferative disorders, including neoplasm, melanoma, lung, colorectal, breast, pancreas, head and neck and other solid tumours; myeloproliferative disorders, such as leukemia, non-Hodgkin lymphoma, leukopenia, thrombocytopenia, angiogenesis disorder, Kaposi's sarcoma; autoimmune/inflammatory disorders, including allergy, inflammatory bowel disease, arthritis, psoriasis and respiratory tract inflammation, asthma, and organ transplant rejection; cardiovascular disorders, including hypertension, oedema, angina, atherosclerosis, thrombosis, sepsis, shock, reperfusion injury, and ischemia; neurological disorders including central nervous system disease, Alzheimer's disease, brain injury, amyotrophic lateral sclerosis, and pain; developmental disorders; metabolic disorders including diabetes mellitus, osteoporosis, and obesity, AIDS and renal disease; infections including viral infection, bacterial infection, fungal infection and parasitic infection and other pathological conditions. Preferably, the disease is one in which the IL-8 like chemokine family is implicated, such as sublethal endotoxaemia, septic shock, microbial infection of the amniotic cavity, Jarish-Herxheimer reaction of relapsing fever, infectious diseases of the central nervous system, acute pancreatitis, ulcerative colitis, empyema, haemolytic uraemic syndrome, meningococcal disease, gastric infection, pertussis, peritonitis, psoriasis, rheumatoid arthritis, sepsis, asthma and glomerulonephritis. These molecules may also be used in the manufacture of a medicament for the treatment of such diseases.

In a ninth aspect, the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide of the first aspect of the invention or the activity of a polypeptide of the first aspect of the invention in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease. Such a method will preferably be carried out *in vitro*. Similar methods may be used for monitoring the therapeutic treatment of disease in a patient, wherein altering the level of expression or activity of a polypeptide or nucleic acid molecule over the period of time

towards a control level is indicative of regression of disease.

A preferred method for detecting polypeptides of the first aspect of the invention comprises the steps of: (a) contacting a ligand, such as an antibody, of the sixth aspect of the invention with a biological sample under conditions suitable for the formation of a
5 ligand-polypeptide complex; and (b) detecting said complex.

A number of different such methods according to the ninth aspect of the invention exist, as the skilled reader will be aware, such as methods of nucleic acid hybridization with short probes, point mutation analysis, polymerase chain reaction (PCR) amplification and methods using antibodies to detect aberrant protein levels. Similar methods may be used
10 on a short or long term basis to allow therapeutic treatment of a disease to be monitored in a patient. The invention also provides kits that are useful in these methods for diagnosing disease.

In a tenth aspect, the invention provides for the use of a polypeptide of the first aspect of the invention as an IL-8 like chemokine. Suitable uses of the polypeptides of the invention
15 as IL-8 like chemokine proteins include use as a regulator of cellular growth, metabolism or differentiation, use as part of a receptor/ligand pair and use as a diagnostic marker for a physiological or pathological condition selected from the list given above.

In an eleventh aspect, the invention provides a pharmaceutical composition comprising a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or
20 third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, in conjunction with a pharmaceutically-acceptable carrier.

In a twelfth aspect, the present invention provides a polypeptide of the first aspect of the
25 invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in the manufacture of a medicament for the diagnosis or treatment of a disease.

30 In a thirteenth aspect, the invention provides a method of treating a disease in a patient comprising administering to the patient a polypeptide of the first aspect of the invention, or

a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention.

For diseases in which the expression of a natural gene encoding a polypeptide of the first
5 aspect of the invention, or in which the activity of a polypeptide of the first aspect of the invention, is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an agonist. Conversely, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient
10 when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an antagonist. Examples of such antagonists include antisense nucleic acid molecules, ribozymes and ligands, such as antibodies.

In a fourteenth aspect, the invention provides transgenic or knockout non-human animals
15 that have been transformed to express higher, lower or absent levels of a polypeptide of the first aspect of the invention. Such transgenic animals are very useful models for the study of disease and may also be used in screening regimes for the identification of compounds that are effective in the treatment or diagnosis of such a disease.

A summary of standard techniques and procedures which may be employed in order to
20 utilise the invention is given below. It will be understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors and reagents described. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and it is not intended that this terminology should limit the scope of the present invention. The extent of the invention is limited only by the terms of
25 the appended claims.

Standard abbreviations for nucleotides and amino acids are used in this specification.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.

30 Such techniques are explained fully in the literature. Examples of particularly suitable texts for consultation include the following: Sambrook Molecular Cloning; A Laboratory

Manual, Second Edition (1989); DNA Cloning, Volumes I and II (D.N Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.I. Freshney ed. 1986); Immobilized Cells and Enzymes
5 (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the Methods in Enzymology series (Academic Press, Inc.), especially volumes 154 & 155; Gene Transfer Vectors for Mammalian Cells (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds. 1987, Academic Press, London); Scopes, (1987) Protein Purification:
10 Principles and Practice, Second Edition (Springer Verlag, N.Y.); and Handbook of Experimental Immunology, Volumes I-IV (D.M. Weir and C. C. Blackwell eds. 1986).

As used herein, the term "polypeptide" includes any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e. peptide isosteres. This term refers both to short chains (peptides and oligopeptides) and to
15 longer chains (proteins).

The polypeptide of the present invention may be in the form of a mature protein or may be a pre-, pro- or prepro- protein that can be activated by cleavage of the pre-, pro- or prepro- portion to produce an active mature polypeptide. In such polypeptides, the pre-, pro- or prepro- sequence may be a leader or secretory sequence or may be a sequence that is
20 employed for purification of the mature polypeptide sequence.

The polypeptide of the first aspect of the invention may form part of a fusion protein. For example, it is often advantageous to include one or more additional amino acid sequences which may contain secretory or leader sequences, pro-sequences, sequences which aid in purification, or sequences that confer higher protein stability, for example during
25 recombinant production. Alternatively or additionally, the mature polypeptide may be fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol).

Polypeptides may contain amino acids other than the 20 gene-encoded amino acids, modified either by natural processes, such as by post-translational processing or by
30 chemical modification techniques which are well known in the art. Among the known modifications which may commonly be present in polypeptides of the present invention are glycosylation, lipid attachment, sulphation, gamma-carboxylation, for instance of

- glutamic acid residues, hydroxylation and ADP-ribosylation. Other potential modifications include acetylation, acylation, amidation, covalent attachment of flavin, covalent attachment of a haeme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulphide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, GPI anchor formation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.
- 10 Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl terminus in a polypeptide, or both, by a covalent modification is common in naturally-occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention.
- 15 The modifications that occur in a polypeptide often will be a function of how the polypeptide is made. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns
- 20 vary between different types of host cell.

The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally-occurring polypeptides (for example purified from cell culture), recombinantly-produced polypeptides (including fusion proteins), synthetically-produced polypeptides or polypeptides that are produced by a combination of

25 these methods.

The functionally-equivalent polypeptides of the first aspect of the invention may be polypeptides that are homologous to the INSP093 or INSP094 polypeptides. Two polypeptides are said to be "homologous", as the term is used herein, if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of

30 the other polypeptide. "Identity" indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is

of a similar type between the sequences. Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

Homologous polypeptides therefore include natural biological variants (for example, allelic variants or geographical variations within the species from which the polypeptides are derived) and mutants (such as mutants containing amino acid substitutions, insertions or deletions) of the INSP093 and INSP094 polypeptides. Such mutants may include polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; or among the aromatic residues Phe and Tyr. Particularly preferred are variants in which several, i.e. between 5 and 10, 1 and 5, 1 and 3, 1 and 2 or just 1 amino acids are substituted, deleted or added in any combination. Especially preferred are silent substitutions, additions and deletions, which do not alter the properties and activities of the protein. Also especially preferred in this regard are conservative substitutions. Such mutants also include polypeptides in which one or more of the amino acid residues includes a substituent group.

Typically, greater than 30% identity between two polypeptides is considered to be an indication of functional equivalence. Preferably, functionally equivalent polypeptides of the first aspect of the invention have a degree of sequence identity with the INSP093 and INSP094 polypeptides, or with active fragments thereof, of greater than 80%. More preferred polypeptides have degrees of identity of greater than 85%, 90%, 95%, 98% or 99%, respectively.

The functionally-equivalent polypeptides of the first aspect of the invention may also be polypeptides which have been identified using one or more techniques of structural alignment. For example, the Inpharmatica Genome Threader technology that forms one

aspect of the search tools used to generate the Biopendium™ search database may be used (see PCT application WO 01/69507) to identify polypeptides of presently-unknown function which, while having low sequence identity as compared to the INSP093 and INSP094 polypeptides, are predicted to be members of the IL-8 like chemokine family, by virtue of sharing significant structural homology with the INSP093 and INSP094 polypeptide sequences. By "significant structural homology" is meant that the Inpharmatica Genome Threader predicts two proteins to share structural homology with a certainty of 10% and above.

The polypeptides of the first aspect of the invention also include fragments of the INSP093 or INSP094 polypeptides and fragments of the functional equivalents of the INSP093 or INSP094 polypeptides, provided that those fragments are members of the IL-8 like chemokine family or have an antigenic determinant in common with the INSP093 or INSP094 polypeptides.

As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of the INSP093 or INSP094 polypeptides or one of their functional equivalents. The fragments should comprise at least *n* consecutive amino acids from the sequence and, depending on the particular sequence, *n* preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small fragments may form an antigenic determinant.

Fragments of the full length INSP093 and INSP094 polypeptides may consist of combinations of 2 or 3 of neighbouring exon sequences in the INSP093 or INSP094 polypeptide sequences, respectively. For example, such combinations include exons A and B of the INSP093 polypeptide. Such fragments are included in the present invention. A further preferred fragment of the invention is that provided in SEQ ID NO:12, namely the fragment of INSP094 cloned herein.

Such fragments may be "free-standing", i.e. not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the fragment of the invention most preferably forms a single continuous region. For instance, certain preferred embodiments relate to a fragment having a pre- and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger

polypeptide.

The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may
5 be employed to isolate or to identify clones expressing the polypeptides of the invention or to purify the polypeptides by affinity chromatography. The antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent to the skilled reader.

The term "immunospecific" means that the antibodies have substantially greater affinity
10 for the polypeptides of the invention than their affinity for other related polypeptides in the prior art. As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')₂ and Fv, which are capable of binding to the antigenic determinant in question. Such antibodies thus bind to the polypeptides of the first aspect of the invention.

15 By "substantially greater affinity" we mean that there is a measurable increase in the affinity for a polypeptide of the invention as compared with the affinity for known secreted proteins.

Preferably, the affinity is at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10³-fold, 10⁴-fold, 10⁵-fold, 10⁶-fold or greater for a polypeptide of the invention than for known
20 secreted proteins such as members of the IL-8 chemokine family of proteins.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or horse, may be immunised with a polypeptide of the first aspect of the invention. The polypeptide used to immunise the animal can be derived by recombinant DNA technology or can be synthesized chemically. If desired, the polypeptide can be conjugated to a carrier
25 protein. Commonly used carriers to which the polypeptides may be chemically coupled include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The coupled polypeptide is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by immunoaffinity chromatography.

30 Monoclonal antibodies to the polypeptides of the first aspect of the invention can also be readily produced by one skilled in the art. The general methodology for making

monoclonal antibodies using hybridoma technology is well known (see, for example, Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985).

- 5 Panels of monoclonal antibodies produced against the polypeptides of the first aspect of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are particularly useful in purification of the individual polypeptides against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the
10 art, and cloned and expressed in appropriate vectors.

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions (see, for example, Liu *et al.*, *Proc. Natl. Acad. Sci. USA*, 84, 3439 (1987)), may also be of use.

- The antibody may be modified to make it less immunogenic in an individual, for example
15 by humanisation (see Jones *et al.*, *Nature*, 321, 522 (1986); Verhoeven *et al.*, *Science*, 239, 1534 (1988); Kabat *et al.*, *J. Immunol.*, 147, 1709 (1991); Queen *et al.*, *Proc. Natl Acad. Sci. USA*, 86, 10029 (1989); Gorman *et al.*, *Proc. Natl Acad. Sci. USA*, 88, 34181 (1991); and Hodgson *et al.*, *Bio/Technology*, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other
20 amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody. The humanised antibody thus closely resembles a human antibody but has the binding ability of the donor antibody.

- In a further alternative, the antibody may be a "bispecific" antibody, that is an antibody
25 having two different antigen binding domains, each domain being directed against a different epitope.

- Phage** display technology may be utilised to select genes which encode antibodies with binding activities towards the polypeptides of the invention either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant
30 antibodies, or from naive libraries (McCafferty, J. *et al.*, (1990), *Nature* 348, 552-554; Marks, J. *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. *et al.*, (1991) *Nature* 352, 624-628).

Antibodies generated by the above techniques, whether polyclonal or monoclonal, have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as
5 a radioisotope, a fluorescent molecule or an enzyme.

Preferred nucleic acid molecules of the second and third aspects of the invention are those which encode a polypeptide sequence as recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10 and SEQ ID NO:12 and functionally equivalent polypeptides. These nucleic acid molecules may be used in the methods and applications
10 described herein. The nucleic acid molecules of the invention preferably comprise at least n consecutive nucleotides from the sequences disclosed herein where, depending on the particular sequence, n is 10 or more (for example, 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

The nucleic acid molecules of the invention also include sequences that are complementary
15 to nucleic acid molecules described above (for example, for antisense or probing purposes).

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance cDNA, synthetic DNA or genomic DNA. Such nucleic acid molecules may be obtained by cloning, by chemical synthetic
20 techniques or by a combination thereof. The nucleic acid molecules can be prepared, for example, by chemical synthesis using techniques such as solid phase phosphoramidite chemical synthesis, from genomic or cDNA libraries or by separation from an organism. RNA molecules may generally be generated by the *in vitro* or *in vivo* transcription of DNA sequences.

25 The nucleic acid molecules may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The term "nucleic acid molecule" also includes analogues of DNA and RNA, such as those containing modified backbones, and peptide nucleic acids (PNA). The term "PNA", as
30 used herein, refers to an antisense molecule or an anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, which preferably ends in lysine. The terminal lysine confers solubility to the

composition. PNAs may be pegylated to extend their lifespan in a cell, where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) *Anticancer Drug Des.* 8:53-63).

A nucleic acid molecule which encodes a polypeptide of this invention may be identical to
5 the coding sequence of one or more of the nucleic acid molecules disclosed herein.

These molecules also may have a different sequence which, as a result of the degeneracy of the genetic code, encodes a polypeptide SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10 or SEQ ID NO:12. Such nucleic acid molecules may include, but are not limited to, the coding sequence for the mature polypeptide by itself; the
10 coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pro-, pre- or prepro- polypeptide sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with further additional, non-coding sequences, including non-coding 5' and 3' sequences, such as the transcribed, non-
15 translated sequences that play a role in transcription (including termination signals), ribosome binding and mRNA stability. The nucleic acid molecules may also include additional sequences which encode additional amino acids, such as those which provide additional functionalities.

The nucleic acid molecules of the second and third aspects of the invention may also
20 encode the fragments or the functional equivalents of the polypeptides and fragments of the first aspect of the invention. Such a nucleic acid molecule may be a naturally-occurring variant such as a naturally-occurring allelic variant, or the molecule may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid
25 molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or insertions. The substitutions, deletions or insertions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative
30 or non-conservative amino acid substitutions, deletions or insertions.

The nucleic acid molecules of the invention can also be engineered, using methods generally known in the art, for a variety of reasons, including modifying the cloning,

processing, and/or expression of the gene product (the polypeptide). DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides are included as techniques which may be used to engineer the nucleotide sequences. Site-directed mutagenesis may be used to insert new restriction sites, alter
5 glycosylation patterns, change codon preference, produce splice variants, introduce mutations and so forth.

Nucleic acid molecules which encode a polypeptide of the first aspect of the invention may be ligated to a heterologous sequence so that the combined nucleic acid molecule encodes a fusion protein. Such combined nucleic acid molecules are included within the second or
10 third aspects of the invention. For example, to screen peptide libraries for inhibitors of the activity of the polypeptide, it may be useful to express, using such a combined nucleic acid molecule, a fusion protein that can be recognised by a commercially-available antibody. A fusion protein may also be engineered to contain a cleavage site located between the sequence of the polypeptide of the invention and the sequence of a heterologous protein so
15 that the polypeptide may be cleaved and purified away from the heterologous protein.

The nucleic acid molecules of the invention also include antisense molecules that are partially complementary to nucleic acid molecules encoding polypeptides of the present invention and that therefore hybridize to the encoding nucleic acid molecules (hybridization). Such antisense molecules, such as oligonucleotides, can be designed to
20 recognise, specifically bind to and prevent transcription of a target nucleic acid encoding a polypeptide of the invention, as will be known by those of ordinary skill in the art (see, for example, Cohen, J.S., Trends in Pharm. Sci., 10, 435 (1989), Okano, J. Neurochem. 56, 560 (1991); O'Connor, J. Neurochem 56, 560 (1991); Lee *et al.*, Nucleic Acids Res 6, 3073 (1979); Cooney *et al.*, Science 241, 456 (1988); Dervan *et al.*, Science 251, 1360 (1991).

25 The term "hybridization" as used here refers to the association of two nucleic acid molecules with one another by hydrogen bonding. Typically, one molecule will be fixed to a solid support and the other will be free in solution. Then, the two molecules may be placed in contact with one another under conditions that favour hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time
30 of hybridization; agitation; agents to block the non-specific attachment of the liquid phase molecule to the solid support (Denhardt's reagent or BLOTTO); the concentration of the molecules; use of compounds to increase the rate of association of molecules (dextran

sulphate or polyethylene glycol); and the stringency of the washing conditions following hybridization (see Sambrook *et al.* [*supra*]).

The inhibition of hybridization of a completely complementary molecule to a target molecule may be examined using a hybridization assay, as known in the art (see, for
5 example, Sambrook *et al.* [*supra*]). A substantially homologous molecule will then compete for and inhibit the binding of a completely homologous molecule to the target molecule under various conditions of stringency, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

10 "Stringency" refers to conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. High stringency hybridisation conditions are defined as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20
15 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65°C. Low stringency conditions involve the hybridisation reaction being carried out at 35°C (see Sambrook *et al.* [*supra*]). Preferably, the conditions used for hybridization are those of high stringency.

Preferred embodiments of this aspect of the invention are nucleic acid molecules that are at
20 least 70% identical over their entire length to a nucleic acid molecule encoding the INSP093 or INSP094 polypeptides and nucleic acid molecules that are substantially complementary to such nucleic acid molecules. Preferably, a nucleic acid molecule according to this aspect of the invention comprises a region that is at least 80% identical over its entire length to such coding sequences, or is a nucleic acid molecule that is
25 complementary thereto. In this regard, nucleic acid molecules at least 90%, preferably at least 95%, more preferably at least 98%, 99% or more identical over their entire length to the same are particularly preferred. Preferred embodiments in this respect are nucleic acid molecules that encode polypeptides which retain substantially the same biological function or activity as the INSP093 or INSP094 polypeptides.

30 The invention also provides a process for detecting a nucleic acid molecule of the invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b)

detecting any such duplexes that are formed.

As discussed additionally below in connection with assays that may be utilised according to the invention, a nucleic acid molecule as described above may be used as a hybridization probe for RNA, cDNA or genomic DNA, in order to isolate full-length
5 cDNAs and genomic clones encoding the INSP093 or INSP094 polypeptides and to isolate cDNA and genomic clones of homologous or orthologous genes that have a high sequence similarity to the gene encoding this polypeptide.

In this regard, the following techniques, among others known in the art, may be utilised and are discussed below for purposes of illustration. Methods for DNA sequencing and
10 analysis are well known and are generally available in the art and may, indeed, be used to practice many of the embodiments of the invention discussed herein. Such methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proof-reading
15 exonucleases such as those found in the ELONGASE Amplification System marketed by Gibco/BRL (Gaithersburg, MD). Preferably, the sequencing process may be automated using machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), the Peltier Thermal Cyler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

20 One method for isolating a nucleic acid molecule encoding a polypeptide with an equivalent function to that of the INSP093 polypeptide is to probe a genomic or cDNA library with a natural or artificially-designed probe using standard procedures that are recognised in the art (see, for example, "Current Protocols in Molecular Biology", Ausubel
25 *et al.* (eds). Greene Publishing Association and John Wiley Interscience, New York, 1989,1992). Probes comprising at least 15, preferably at least 30, and more preferably at least 50, contiguous bases that correspond to, or are complementary to, nucleic acid sequences from the appropriate encoding gene (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9), are particularly useful probes. Such probes may be labelled with an analytically-detectable reagent to facilitate their identification. Useful
30 reagents include, but are not limited to, radioisotopes, fluorescent dyes and enzymes that are capable of catalysing the formation of a detectable product. Using these probes, the ordinarily skilled artisan will be capable of isolating complementary copies of genomic

DNA, cDNA or RNA polynucleotides encoding proteins of interest from human, mammalian or other animal sources and screening such sources for related sequences, for example, for additional members of the family, type and/or subtype.

- In many cases, isolated cDNA sequences will be incomplete, in that the region encoding the polypeptide will be cut short, normally at the 5' end. Several methods are available to obtain full length cDNAs, or to extend short cDNAs. Such sequences may be extended utilising a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed is based on the method of Rapid Amplification of cDNA Ends (RACE; see, for example, Frohman *et al.*, PNAS USA 85, 8998-9002, 1988). Recent modifications of this technique, exemplified by the MarathonTM technology (Clontech Laboratories Inc.), for example, have significantly simplified the search for longer cDNAs. A slightly different technique, termed "restriction-site" PCR, uses universal primers to retrieve unknown nucleic acid sequence adjacent a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). Inverse PCR may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.* (1988) Nucleic Acids Res. 16:8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.* (1991) PCR Methods Applic., 1, 111-119). Another method which may be used to retrieve unknown sequences is that of Parker, J.D. *et al.* (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinderTM libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.
- When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

In one embodiment of the invention, the nucleic acid molecules of the present invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is

specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important step in the confirmatory correlation of those sequences with the gene-associated disease. Once a sequence has been mapped to a precise
5 chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance
10 of physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleic acid molecule may also be used to
15 detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

The nucleic acid molecules of the present invention are also valuable for tissue localisation. Such techniques allow the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques
20 include *in situ* hybridization techniques and nucleotide amplification techniques, such as PCR. Results from these studies provide an indication of the normal functions of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable insights into the role of mutant polypeptides in disease. Such inappropriate expression may
25 be of a temporal, spatial or quantitative nature.

Gene silencing approaches may also be undertaken to down-regulate endogenous expression of a gene encoding a polypeptide of the invention. RNA interference (RNAi) (Elbashir, SM *et al.*, Nature 2001, 411, 494-498) is one method of sequence specific post-transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are
30 synthesised *in vitro* and introduced into a cell. The sequence specific binding of these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression.

Efficacy of the gene silencing approaches assessed above may be assessed through the measurement of polypeptide expression (for example, by Western blotting), and at the RNA level using TaqMan-based methodologies.

5 The vectors of the present invention comprise nucleic acid molecules of the invention and may be cloning or expression vectors. The host cells of the invention, which may be transformed, transfected or transduced with the vectors of the invention may be prokaryotic or eukaryotic.

10 The polypeptides of the invention may be prepared in recombinant form by expression of their encoding nucleic acid molecules in vectors contained within a host cell. Such expression methods are well known to those of skill in the art and many are described in detail by Sambrook *et al.* (*supra*) and Fernandez & Hoeffler (1998, eds. "Gene expression systems. Using nature for the art of expression". Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto).

15 Generally, any system or vector that is suitable to maintain, propagate or express nucleic acid molecules to produce a polypeptide in the required host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those described in Sambrook *et al.*, (*supra*). Generally, the encoding gene can be placed under the control of a control element such as a promoter, ribosome binding site (for bacterial expression) and, optionally, an
20 operator, so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the transformed host cell.

Examples of suitable expression systems include, for example, chromosomal, episomal and virus-derived systems, including, for example, vectors derived from: bacterial plasmids, bacteriophage, transposons, yeast episomes, insertion elements, yeast chromosomal
25 elements, viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, or combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, including cosmids and phagemids. Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. The
30 vector pCR4-TOPO-INSP094 (Figure 7), pENTR-INSP094-6HIS (Figure 11), pEAK12d-INSP094-6HIS (Figure 12) and pDEST12.2-INSP094-6HIS (Figure 13) are preferred examples of suitable vectors for use in accordance with the aspects of this invention

relating to INSP094.

Particularly suitable expression systems include microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (for example, baculovirus); plant cell systems transformed with virus expression vectors (for example, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (for example, Ti or pBR322 plasmids); or animal cell systems. Cell-free translation systems can also be employed to produce the polypeptides of the invention.

- 10 Introduction of nucleic acid molecules encoding a polypeptide of the present invention into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, (*supra*). Particularly suitable methods include calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, 15 electroporation, transduction, scrape loading, ballistic introduction or infection (see Sambrook *et al.*, 1989 [*supra*]; Ausubel *et al.*, 1991 [*supra*]; Spector, Goldman & Leinwald, 1998). In eukaryotic cells, expression systems may either be transient (for example, episomal) or permanent (chromosomal integration) according to the needs of the system.
- 20 The encoding nucleic acid molecule may or may not include a sequence encoding a control sequence, such as a signal peptide or leader sequence, as desired, for example, for secretion of the translated polypeptide into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals. Leader sequences can 25 be removed by the bacterial host in post-translational processing.

In addition to control sequences, it may be desirable to add regulatory sequences that allow for regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those which cause the expression of a gene to be increased or decreased in response to a chemical or physical stimulus, including the 30 presence of a regulatory compound or to various temperature or metabolic conditions. Regulatory sequences are those non-translated regions of the vector, such as enhancers, promoters and 5' and 3' untranslated regions. These interact with host cellular proteins to

carry out transcription and translation. Such regulatory sequences may vary in their strength and specificity. Depending on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible
5 promoters such as the hybrid lacZ promoter of the Bluescript phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (for example, heat shock, RUBISCO and storage protein genes) or from plant viruses (for example, viral promoters or leader sequences) may be cloned into
10 the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

An expression vector is constructed so that the particular nucleic acid coding sequence is
15 located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the regulatory sequences being such that the coding sequence is transcribed under the "control" of the regulatory sequences, i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence. In some cases it may be necessary to modify the sequence so that it
20 may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame.

The control sequences and other regulatory sequences may be ligated to the nucleic acid coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector that already contains the control sequences and an
25 appropriate restriction site.

For long-term, high-yield production of a recombinant polypeptide, stable expression is preferred. For example, cell lines which stably express the polypeptide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate
30 vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and

recovery of cells that successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalised cell lines available from the American Type Culture Collection (ATCC) including, but not limited to, Chinese hamster ovary (CHO), HeLa, baby hamster kidney (BHK), monkey kidney (COS), C127, 3T3, BHK, HEK 293, Bowes melanoma and human hepatocellular carcinoma (for example Hep G2) cells and a number of other cell lines.

10 In the baculovirus system, the materials for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA (the "MaxBac" kit). These techniques are generally known to those skilled in the art and are described fully in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Particularly suitable host cells for use in this system include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells.

There are many plant cell culture and whole plant genetic expression systems known in the art. Examples of suitable plant cellular genetic expression systems include those described in US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30, 3861-3863 (1991).

In particular, all plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be utilised, so that whole plants are recovered which contain the transferred gene. Practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugar cane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Examples of particularly preferred bacterial host cells include *streptococci*, *staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells.

Examples of particularly suitable host cells for fungal expression include yeast cells (for example, *S. cerevisiae*) and *Aspergillus* cells.

30 Any number of selection systems are known in the art that may be used to recover transformed cell lines. Examples include the herpes simplex virus thymidine kinase

(Wigler, M. *et al.* (1977) Cell 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1980) Cell 22:817-23) genes that can be employed in tk⁻ or apt⁺ cells, respectively.

Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dihydrofolate reductase (DHFR) that confers resistance to methotrexate (Wigler, M. *et al.* (1980) Proc. Natl. Acad. Sci. 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al.* (1981) J. Mol. Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. Additional selectable genes have been described, examples of which will be clear to those of skill in the art.

Although the presence or absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the relevant sequence is inserted within a marker gene sequence, transformed cells containing the appropriate sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain a nucleic acid sequence encoding a polypeptide of the invention and which express said polypeptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassays, for example, fluorescence activated cell sorting (FACS) or immunoassay techniques (such as the enzyme-linked immunosorbent assay [ELISA] and radioimmunoassay [RIA]), that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein (see Hampton, R. *et al.* (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. *et al.* (1983) J. Exp. Med, 158, 1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting sequences related to nucleic acid molecules encoding polypeptides of the present invention include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled polynucleotide. Alternatively, the sequences encoding the polypeptide of the invention may be cloned into

a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

10 Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells, or by germ line therapy to incorporate heritable modifications. Such transgenic animals may be particularly useful in the generation of animal models for drug molecules effective
15 as modulators of the polypeptides of the present invention.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite
20 chromatography and lectin chromatography. High performance liquid chromatography is particularly useful for purification. Well known techniques for refolding proteins may be employed to regenerate an active conformation when the polypeptide is denatured during isolation and or purification.

Specialised vector constructions may also be used to facilitate purification of proteins, as
25 desired, by joining sequences encoding the polypeptides of the invention to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Examples of such purification-facilitating domains include metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobulin, and the
30 domain utilised in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the

polypeptide of the invention may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing the polypeptide of the invention fused to several histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilised metal ion affinity chromatography as described in Porath, J. *et al.* (1992), Prot. Exp. Purif. 3: 263-281) while the thioredoxin or enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. *et al.* (1993; DNA Cell Biol. 12:441-453).

If the polypeptide is to be expressed for use in screening assays, generally it is preferred that it be produced at the surface of the host cell in which it is expressed. In this event, the host cells may be harvested prior to use in the screening assay, for example using techniques such as fluorescence activated cell sorting (FACS) or immunoaffinity techniques. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the expressed polypeptide. If polypeptide is produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

The polypeptide of the invention can be used to screen libraries of compounds in any of a variety of drug screening techniques. Such compounds may activate (agonise) or inhibit (antagonise) the level of expression of the gene or the activity of the polypeptide of the invention and form a further aspect of the present invention. Preferred compounds are effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

Agonist or antagonist compounds may be isolated from, for example, cells, cell-free preparations, chemical libraries or natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors or structural or functional mimetics. For a suitable review of such screening techniques, see Coligan *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991).

Compounds that are most likely to be good antagonists are molecules that bind to the polypeptide of the invention without inducing the biological effects of the polypeptide upon binding to it. Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to the polypeptide of the invention and thereby inhibit or extinguish its activity. In this fashion, binding of the polypeptide to normal

cellular binding molecules may be inhibited, such that the normal biological activity of the polypeptide is prevented.

The polypeptide of the invention that is employed in such a screening technique may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly.

5 In general, such screening procedures may involve using appropriate cells or cell membranes that express the polypeptide that are contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The functional response of the cells contacted with the test compound is then compared with control cells that were not contacted with the test compound. Such an assay may assess whether the test
10 compound results in a signal generated by activation of the polypeptide, using an appropriate detection system. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist in the presence of the test compound is observed.

A preferred method for identifying an agonist or antagonist compound of a polypeptide of
15 the present invention comprises:

(a) contacting a cell expressing on the surface thereof the polypeptide according to the first aspect of the invention, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the
20 polypeptide; and

(b) determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.

A further preferred method for identifying an agonist or antagonist of a polypeptide of the
25 invention comprises:

(a) contacting a cell expressing on the surface thereof the polypeptide, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and
30 (b) determining whether the compound binds to and activates or inhibits the polypeptide by comparing the level of a signal generated from the interaction of the compound with the

polypeptide with the level of a signal in the absence of the compound.

In further preferred embodiments, the general methods that are described above may further comprise conducting the identification of agonist or antagonist in the presence of labelled or unlabelled ligand for the polypeptide.

- 5 In another embodiment of the method for identifying an agonist or antagonist of a polypeptide of the present invention comprises:

determining the inhibition of binding of a ligand to cells which have a polypeptide of the invention on the surface thereof, or to cell membranes containing such a polypeptide, in the presence of a candidate compound under conditions to permit binding to the
10 polypeptide, and determining the amount of ligand bound to the polypeptide. A compound capable of causing reduction of binding of a ligand is considered to be an agonist or antagonist. Preferably the ligand is labelled.

More particularly, a method of screening for a polypeptide antagonist or agonist compound comprises the steps of:

- 15 (a) incubating a labelled ligand with a whole cell expressing a polypeptide according to the invention on the cell surface, or a cell membrane containing a polypeptide of the invention,
(b) measuring the amount of labelled ligand bound to the whole cell or the cell membrane;
(c) adding a candidate compound to a mixture of labelled ligand and the whole cell or the cell membrane of step (a) and allowing the mixture to attain equilibrium;
20 (d) measuring the amount of labelled ligand bound to the whole cell or the cell membrane after step (c); and
(e) comparing the difference in the labelled ligand bound in step (b) and (d), such that the compound which causes the reduction in binding in step (d) is considered to be an agonist or antagonist.
- 25 The INSP094 polypeptides may also be found to modulate immune and/or nervous system cell proliferation and differentiation in a dose-dependent manner in the above-described assays. Thus, the "functional equivalents" of the INSP094 polypeptides include polypeptides that exhibit any of the same growth and differentiation regulating activities in the above-described assays in a dose-dependent manner. Although the degree of
30 dose-dependent activity need not be identical to that of the INSP094 polypeptides,

preferably the "functional equivalents" will exhibit substantially similar dose-dependence in a given activity assay compared to the INSP094 polypeptides.

In certain of the embodiments described above, simple binding assays may be used, in which the adherence of a test compound to a surface bearing the polypeptide is detected by
5 means of a label directly or indirectly associated with the test compound or in an assay involving competition with a labelled competitor. In another embodiment, competitive drug screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that
10 possesses specific binding affinity for the polypeptide.

Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be
15 used to search for compounds that may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured. Examples of IL-8 assays include Pelikine compact Human IL-8 ELISA (Research Diagnostics), IL-8 TiterZyme® Immunoassay kit (Assay Designs, Inc.) and Immulite® IL-
20 8 test (Diagnostic Products Corporation).

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different small test compounds are synthesised on a solid substrate, which may then be reacted with
25 the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

The polypeptide of the invention may be used to identify membrane-bound or soluble
30 receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that

facilitates its detection or purification, and incubated with a source of the putative receptor (for example, a composition of cells, cell membranes, cell supernatants, tissue extracts, or bodily fluids). The efficacy of binding may be measured using biophysical techniques such as surface plasmon resonance and spectroscopy. Binding assays may be used for the
5 purification and cloning of the receptor, but may also identify agonists and antagonists of the polypeptide, that compete with the binding of the polypeptide to its receptor. Standard methods for conducting screening assays are well understood in the art.

The invention also includes a screening kit useful in the methods for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, that are described above.

10 The invention includes the agonists, antagonists, ligands, receptors, substrates and enzymes, and other compounds which modulate the activity or antigenicity of the polypeptide of the invention discovered by the methods that are described above.

The invention also provides pharmaceutical compositions comprising a polypeptide, nucleic acid, ligand or compound of the invention in combination with a suitable
15 pharmaceutical carrier. These compositions may be suitable as therapeutic or diagnostic reagents, as vaccines, or as other immunogenic compositions, as outlined in detail below.

According to the terminology used herein, a composition containing a polypeptide, nucleic acid, ligand or compound [X] is "substantially free of" impurities [herein, Y] when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least
20 about 90% by weight of the total of X+Y in the composition, more preferably at least about 95%, 98% or even 99% by weight.

The pharmaceutical compositions should preferably comprise a therapeutically effective amount of the polypeptide, nucleic acid molecule, ligand, or compound of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a
25 therapeutic agent needed to treat, ameliorate, or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, for example, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route
30 of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. This amount can be determined by routine experimentation
5 and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg. Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier, for
10 administration of a therapeutic agent. Such carriers include antibodies and other polypeptides, genes and other therapeutic agents such as liposomes, provided that the carrier does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolised macromolecules such as proteins,
15 polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulphates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough
20 discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in
25 such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be
30 treated.

The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-

arterial, intramedullary, intrathecal, intraventricular, transdermal or transcutaneous applications (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal means. Gene guns or hypodermic sprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the
5 therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the
10 interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

If the activity of the polypeptide of the invention is in excess in a particular disease state, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as described above, along with a pharmaceutically
15 acceptable carrier in an amount effective to inhibit the function of the polypeptide, such as by blocking the binding of ligands, substrates, enzymes, receptors, or by inhibiting a second signal, and thereby alleviating the abnormal condition. Preferably, such antagonists are antibodies. Most preferably, such antibodies are chimeric and/or humanised to minimise their immunogenicity, as described previously.

20 In another approach, soluble forms of the polypeptide that retain binding affinity for the ligand, substrate, enzyme, receptor, in question, may be administered. Typically, the polypeptide may be administered in the form of fragments that retain the relevant portions.

In an alternative approach, expression of the gene encoding the polypeptide can be inhibited using expression blocking techniques, such as the use of antisense nucleic acid
25 molecules (as described above), either internally generated or separately administered. Modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions (signal sequence, promoters, enhancers and introns) of the gene encoding the polypeptide. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple
30 helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee,

J.E. *et al.* (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Such oligonucleotides may be administered or may be
5 generated *in situ* from expression *in vivo*.

In addition, expression of the polypeptide of the invention may be prevented by using ribozymes specific to its encoding mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, *et al.*, Curr. Opin. Struct. Biol (1996) 6(4), 527-33). Synthetic ribozymes can be designed to specifically
10 cleave mRNAs at selected positions thereby preventing translation of the mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesised with non-natural backbones, for example, 2'-O-methyl RNA, to provide protection from ribonuclease degradation and may contain modified
15 bases.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent
20 in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine and butosine, as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine and uridine which are not as easily recognised by endogenous endonucleases.

For treating abnormal conditions related to an under-expression of the polypeptide of the
25 invention and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound that activates the polypeptide, i.e., an agonist as described above, to alleviate the abnormal condition. Alternatively, a therapeutic amount of the polypeptide in combination with a suitable pharmaceutical carrier may be administered to restore the relevant physiological balance of
30 polypeptide.

Gene therapy may be employed to effect the endogenous production of the polypeptide by the relevant cells in the subject. Gene therapy is used to treat permanently the inappropriate

production of the polypeptide by replacing a defective gene with a corrected therapeutic gene.

Gene therapy of the present invention can occur *in vivo* or *ex vivo*. *Ex vivo* gene therapy requires the isolation and purification of patient cells, the introduction of a therapeutic
5 gene and introduction of the genetically altered cells back into the patient. In contrast, *in vivo* gene therapy does not require isolation and purification of a patient's cells.

The therapeutic gene is typically "packaged" for administration to a patient. Gene delivery vehicles may be non-viral, such as liposomes, or replication-deficient viruses, such as adenovirus as described by Berkner, K.L., in Curr. Top. Microbiol. Immunol., 158, 39-66
10 (1992) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr. Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Patent No. 5,252,479. For example, a nucleic acid molecule encoding a polypeptide of the invention may be engineered for expression in a replication-defective retroviral vector. This expression construct may then be isolated and introduced into a packaging cell transduced with a
15 retroviral plasmid vector containing RNA encoding the polypeptide, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo* (see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular
20 Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

In situations in which the polypeptides or nucleic acid molecules of the invention are disease-causing agents, the invention provides that they can be used in vaccines to raise
25 antibodies against the disease causing agent.

Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above, which include
30 any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a

bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, and other pathogens.

Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, 5 intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

10 The vaccine formulations of the invention may be presented in unit-dose or multi-dose containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

15 Genetic delivery of antibodies that bind to polypeptides according to the invention may also be effected, for example, as described in International patent application WO98/55607.

The technology referred to as jet injection (see, for example, www.powderject.com) may also be useful in the formulation of vaccine compositions.

20 A number of suitable methods for vaccination and vaccine delivery systems are described in International patent application WO00/29428.

This invention also relates to the use of nucleic acid molecules according to the present invention as diagnostic reagents. Detection of a mutated form of the gene characterised by the nucleic acid molecules of the invention which is associated with a dysfunction will 25 provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acid molecules for diagnosis may be obtained from a subject's cells, such as from 30 blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, ligase chain

reaction (LCR), strand displacement amplification (SDA), or other amplification techniques (see Saiki *et al.*, Nature, 324, 163-166 (1986); Bej, *et al.*, Crit. Rev. Biochem. Molec. Biol., 26, 301-334 (1991); Birkenmeyer *et al.*, J. Virol. Meth., 35, 117-126 (1991); Van Brunt, J., Bio/Technology, 8, 291-294 (1990)) prior to analysis.

- 5 In one embodiment, this aspect of the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to the invention and comparing said level of expression to a control level, wherein a level that is different to said control level is indicative of disease. The method may comprise the steps of:
- 10 a)contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule of the invention and the probe;
- b)contacting a control sample with said probe under the same conditions used in step a);
- c)and detecting the presence of hybrid complexes in said samples;
- 15 wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.

A further aspect of the invention comprises a diagnostic method comprising the steps of:

- a)obtaining a tissue sample from a patient being tested for disease;
- b)isolating a nucleic acid molecule according to the invention from said tissue sample; and
- 20 c)diagnosing the patient for disease by detecting the presence of a mutation in the nucleic acid molecule which is associated with disease.

To aid the detection of nucleic acid molecules in the above-described methods, an amplification step, for example using PCR, may be included.

- Deletions and insertions can be detected by a change in the size of the amplified product in
- 25 comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labelled RNA of the invention or alternatively, labelled antisense DNA sequences of the invention. Perfectly-matched sequences can be distinguished from mismatched duplexes by RNase digestion or by assessing differences in melting temperatures. The presence or absence of the mutation in the patient may be detected by
- 30 contacting DNA with a nucleic acid probe that hybridises to the DNA under stringent

conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence
5 of a disease-associated mutation in the corresponding portion of the DNA strand.

Such diagnostics are particularly useful for prenatal and even neonatal testing.

Point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by other well-known techniques, such as direct DNA sequencing or single-strand conformational polymorphism, (see Orita *et al.*, Genomics, 5, 874-879
10 (1989)). For example, a sequencing primer may be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabelled nucleotides or by automatic sequencing procedures with fluorescent-tags. Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is
15 greatly enhanced when combined with PCR. Further, point mutations and other sequence variations, such as polymorphisms, can be detected as described above, for example, through the use of allele-specific oligonucleotides for PCR amplification of sequences that differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic
20 mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401).

25 In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by *in situ* analysis (see, for example, Keller *et al.*, DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. Fluorescence *in*
30 *situ* hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, Science, 250, 559-562 (1990), and Trask *et al.*, Trends, Genet., 7, 149-154 (1991)).

In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee *et al.*, Science (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in PCT application WO95/11995 (Chee *et al.*); Lockhart, D. J. *et al.* (1996) Nat. Biotech. 14: 1675-1680); and Schena, M. *et al.* (1996) Proc. Natl. Acad. Sci. 93: 10614-10619).

10 Oligonucleotide pairs may range from two to over one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/25116 (Baldeschweiler *et al.*). In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and over one million which lends itself to the efficient use of commercially-available instrumentation.

In addition to the methods discussed above, diseases may be diagnosed by methods comprising determining, from a sample derived from a subject, an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

Assay techniques that can be used to determine levels of a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art and are

discussed in some detail above (including radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays). This aspect of the invention provides a diagnostic method which comprises the steps of: (a) contacting a ligand as described above with a biological sample under conditions suitable for the formation of a ligand-
5 polypeptide complex; and (b) detecting said complex.

Protocols such as ELISA (as previously described), RIA, and FACS for measuring polypeptide levels may additionally provide a basis for diagnosing altered or abnormal levels of polypeptide expression. Normal or standard values for polypeptide expression are established by combining body fluids or cell extracts taken from normal mammalian
10 subjects, preferably humans, with antibody to the polypeptide under conditions suitable for complex formation. The amount of standard complex formation may be quantified by various methods, such as by photometric means.

Antibodies which specifically bind to a polypeptide of the invention may be used for the diagnosis of conditions or diseases characterised by expression of the polypeptide, or in
15 assays to monitor patients being treated with the polypeptides, nucleic acid molecules, ligands and other compounds of the invention. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for the polypeptide include methods that utilise the antibody and a label to detect the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be
20 used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject
25 values establishes the parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

30 A diagnostic kit of the present invention may comprise:

(a) a nucleic acid molecule of the present invention;

(b) a polypeptide of the present invention; or

(c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule
5 according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic
10 acid molecules, at least one of which may be a nucleic acid molecule according to the invention.

To detect polypeptide according to the invention, a diagnostic kit may comprise one or more antibodies that bind to a polypeptide according to the invention; and a reagent useful for the detection of a binding reaction between the antibody and the polypeptide.

15 Such kits will be of use in diagnosing a disease or susceptibility to disease in which members of the IL-8 like chemokine family are implicated. Such diseases may include cell proliferative disorders, including neoplasm, melanoma, lung, colorectal, breast, pancreas, head and neck and other solid tumours; myeloproliferative disorders, such as leukemia, non-Hodgkin lymphoma, leukopenia, thrombocytopenia, angiogenesis disorder, Kaposi's
20 sarcoma; autoimmune/inflammatory disorders, including allergy, inflammatory bowel disease, arthritis, psoriasis and respiratory tract inflammation, asthma, and organ transplant rejection; cardiovascular disorders, including hypertension, oedema, angina, atherosclerosis, thrombosis, sepsis, shock, reperfusion injury, and ischemia; neurological disorders including central nervous system disease, Alzheimer's disease, brain injury,
25 amyotrophic lateral sclerosis, and pain; developmental disorders; metabolic disorders including diabetes mellitus, osteoporosis, and obesity, AIDS and renal disease; infections including viral infection, bacterial infection, fungal infection and parasitic infection and other pathological conditions. Preferably, the diseases are those in which members of the IL-8 like chemokine family are implicated, such as sublethal endotoxaemia, septic shock,
30 microbial infection of the amniotic cavity, Jarish-Herxheimer reaction of relapsing fever, infectious diseases of the central nervous system, acute pancreatitis, ulcerative colitis, empyaema, haemolytic uraemic syndrome, meningococcal disease, gastric infection,

paertussis, peritonitis, psoriasis, rheumatoid arthritis, sepsis, asthma and glomerulonephritis.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to the INSP093 and INSP094
5 polypeptides.

It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of the Figures

Figure 1: Stand alone Genome Threader results for INSP093 partial polypeptide sequence
10 (SEQ ID NO: 6).

Figure 2: Alignment between INSP093 partial polypeptide sequence (SEQ ID NO:6) and the top hit, iqq7 (Stroma cell-derived factor-1alpha).

Figure 3: Stand alone Genome Threader results for INSP094 partial polypeptide sequence (SEQ ID NO: 10).

15 **Figure 4:** Alignment between INSP094 partial polypeptide sequence (SEQ ID NO: 10) and the top hit, 1hum (Human macrophage inflammatory protein 1 beta).

Figure 5: Nucleotide sequence of INSP094 with translation.

Figure 6: Nucleotide sequence with translation of PCR product cloned using primers INSP094-CP1 and INSP094-CP2.

20 **Figure 7:** Map of pCR4-TOPO-INSP094

Figure 8: Map of pDONR 221

Figure 9: Map of expression vector pEAK12d

Figure 10: Map of Expression vector pDEST12.2

Figure 11: Map of pENTR-INSP094-6HIS

25 **Figure 12:** Map of pEAK12d-INSP094-6HIS

Figure 13: Map of pDEST12.2-INSP094-6HIS

Examples

Example 1: INSP093 Genome Threader results

The INSP093 partial polypeptide sequence (SEQ ID NO:8) was searched against PDB structures from the Biopendium database using Genome Threader™. The top match
5 (Figure 1) was for a PDB entry annotated as a CXC-chemokine. Furthermore, Genome Threader™ predicts that the INSP093 polypeptide folds in the same way as the top hit with a high degree of confidence (65%). Figure 2 shows the actual sequence-structure alignment generated by Genome Threader™ of INSP093 partial polypeptide sequence (SEQ ID NO:8) against the top structure, 1qg7 (Stroma cell derived factor-1alpha).

10

Example 2: INSP094 Genome Threader results

The INSP094 partial polypeptide sequence (SEQ ID NO:10) was searched against PDB structures from the Biopendium database using Genome Threader™. The top match
15 (Figure 3) was for a PDB entry annotated as a CC-chemokine. Furthermore, Genome Threader™ predicts that the INSP094 polypeptide folds in the same way as the top hit with a high degree of confidence (65%). Figure 4 shows the actual sequence-structure alignment generated by Genome Threader™ of INSP094 partial polypeptide sequence (SEQ ID NO:10) against the top structure, 1hum (*Homo sapiens* macrophage inflammatory protein 1 beta).

20

Example 3: Cloning of INSP094

1. PCR of INSP094 from genomic DNA

A 264 bp PCR product containing a partial region of the INSP094 predicted coding sequence was amplified from genomic DNA using gene-specific cloning primers
25 (INSP094-CP1 and INSP094-CP2, Table 1, Figures 5 and 6). The PCR was performed in a ~~final~~ volume of 50µl containing 1X AmpliTaq™ buffer, 200µM dNTPs, 50 pmoles of each cloning primer, 2.5 units of AmpliTaq™ (Perkin Elmer) and 100 ng of genomic DNA (Novagen Inc.) using an MJ Research DNA Engine, programmed as follows: 94°C, 2 min; 30 cycles of 94°C, 30 sec, 51°C, 30 sec, and 72°C, 30 sec; followed by 1 cycle at 72°C for
30 7 min and a holding cycle at 4°C.

The amplification products were visualized on 0.8% agarose gel in 1 X TAE buffer (Invitrogen) and a PCR product migrating at the predicted molecular mass was purified from the gel using the Wizard PCR Preps DNA Purification System (Promega). The PCR product was eluted in 50µl of sterile water and subcloned directly.

5

2. Gene specific cloning primers for PCR

A pair of PCR primers having a length of between 18 and 25 bases were designed for amplifying the coding sequence of the virtual cDNA using Primer Designer Software (Scientific & Educational Software, PO Box 72045, Durham, NC 27722-2045, USA). PCR
10 primers were optimized to have a T_m close to $55 \pm 10^\circ\text{C}$ and a GC content of 40-60%. Primers were selected which had high selectivity for the target sequence (little or no specific priming).

3. Subcloning of PCR Products

15 PCR products were subcloned into the topoisomerase I modified cloning vector (pCR4-TOPO) using the TOPO cloning kit purchased from the Invitrogen Corporation using the conditions specified by the manufacturer. Briefly, 4µl of gel purified PCR product was incubated for 15 min at room temperature with 1µl of TOPO vector and 1µl salt solution. The reaction mixture was then transformed into *E. coli* strain TOP10 (Invitrogen) as
20 follows: a 50µl aliquot of One Shot TOP10 cells was thawed on ice and 2µl of TOPO reaction was added. The mixture was incubated for 15 min on ice and then heat shocked by incubation at 42°C for exactly 30s. Samples were returned to ice and 250µl of warm SOC media (room temperature) was added. Samples were incubated with shaking (220 rpm) for 1h at 37°C . The transformation mixture was then plated on L-broth (LB) plates containing
25 ampicillin (100µg/ml) and incubated overnight at 37°C . Ampicillin resistant colonies containing inserts were identified by colony PCR.

4. Colony PCR

Colonies were inoculated into 50µl sterile water using a sterile toothpick. A 10µl aliquot of
30 the inoculum was then subjected to PCR in a total reaction volume of 20µl as described above, except the primers used were T7 and T3. The cycling conditions were as follows:

94°C, 2 min; 30 cycles of 94°C, 30 sec, 48°C, 30 sec and 72°C, 30 sec. Samples were then maintained at 4°C (holding cycle) before further analysis.

PCR reaction products were analyzed on 1% agarose gel in 1 X TAE buffer. Colonies which gave the expected PCR product size (approximately 264 bp cDNA + 105 bp due to the multiple cloning site or MCS) were grown up overnight at 37°C in 5ml L-Broth (LB) containing ampicillin (100µg /ml), with shaking (220 rpm).

5. Plasmid DNA preparation and sequencing

Miniprep plasmid DNA was prepared from 5ml cultures using a Qiaprep Turbo 9600 robotic system (Qiagen) or Wizard Plus SV Minipreps kit (Promega cat. no. 1460) according to the manufacturer's instructions. Plasmid DNA was eluted in 100µl of sterile water. The DNA concentration was measured using an Eppendorf BO photometer. Plasmid DNA (200-500ng) was subjected to DNA sequencing with T7 and T3 primers using the BigDyeTerminator system (Applied Biosystems cat. no. 4390246) according to the manufacturer's instructions. The primer sequences are shown in Table 1. Sequencing reactions were purified using Dye-Ex columns (Qiagen) or Montage SEQ 96 cleanup plates (Millipore cat. no. LSKS09624) then analyzed on an Applied Biosystems 3700 sequencer.

Sequence analysis identified one clone which contained 100% match to the predicted INSP094 sequence. The sequence of this cloned fragment is shown in Figure 6. The plasmid map of the cloned PCR product (pCR4-TOPO-INSP094) (plasmid ID.13736) is shown in Figure 7.

Example 4: Construction of a plasmid for the expression of INSP094 in HEK293/EBNA cells.

A pCR4-TOPO clone containing the full coding sequence (ORF) of INSP094 identified by DNA sequencing (pCR4-TOPO-INSP094, plasmid ID. 13736) (figure 7) was then used to subclone the insert into the mammalian cell expression vectors pEAK12d (figure 9) and pDEST12.2 (figure 10) using the Gateway™ cloning methodology (Invitrogen).

1. Generation of Gateway compatible INSP094 ORF fused to an in frame 6HIS tag sequence.

The first stage of the Gateway cloning process involves a two step PCR reaction which generates the ORF of INSP094 flanked at the 5' end by an attB1 recombination site and Kozak sequence, and flanked at the 3' end by a sequence encoding an in frame 6 histidine (6HIS) tag, a stop codon and the attB2 recombination site (Gateway compatible cDNA). The first PCR reaction (in a final volume of 50µl) contains: 1.5µl of pCR4-TOPO-INSP094 (plasmid ID 13736), 1.5µl dNTPs (10 mM), 10µl of 10X Pfx polymerase buffer, 1µl MgSO₄ (50mM), 0.5µl each of gene specific primer (100µM) (INSP094-EX1 and INSP094-EX2), 2.5µl 10X Enhancer™ solution (Invitrogen) and 1µl Platinum Pfx DNA polymerase (Invitrogen). The PCR reaction was performed using an initial denaturing step of 95°C for 2 min, followed by 25 cycles of 94°C for 15s; 55°C for 30s and 68°C for 2 min; and a holding cycle of 4°C. The amplification products were visualized on 0.8% agarose gel in 1 X TAE buffer (Invitrogen) and a product migrating at the predicted molecular mass was purified from the gel using the Wizard PCR Preps DNA Purification System (Promega) and recovered in 50µl sterile water according to the manufacturer's instructions.

The second PCR reaction (in a final volume of 50µl) contained 10µl purified PCR 1 product, 1.5µl dNTPs (10mM), 5µl of 10X Pfx polymerase buffer, 1µl MgSO₄ (50mM), 0.5µl of each Gateway conversion primer (100µM) (GCP forward and GCP reverse) and 0.5µl of Platinum Pfx DNA polymerase. The conditions for the 2nd PCR reaction were: 95°C for 1 min; 4 cycles of 94°C, 15 sec; 50°C, 30 sec and 68°C for 3 min; 25 cycles of 94°C, 15 sec; 55°C, 30 sec and 68°C, 3 min; followed by a holding cycle of 4°C. PCR products were gel purified using the Wizard PCR prep DNA purification system (Promega) according to the manufacturer's instructions.

2. Subcloning of Gateway compatible INSP094 ORF into Gateway entry vector pDONR221 and expression vectors pEAK12d and pDEST12.2

The second stage of the Gateway cloning process involves subcloning of the Gateway modified PCR product into the Gateway entry vector pDONR221 (Invitrogen, figure 8) as follows: 5µl of purified product from PCR2 were incubated with 1µl pDONR221 vector (0.15µg/µl), 2µl BP buffer and 1.5µl of BP clonase enzyme mix (Invitrogen) in a final volume of 10µl at RT for 1h. The reaction was stopped by addition of proteinase K (2µg)

and incubated at 37°C for a further 10 min. An aliquot of this reaction (2µl) was used to transform *E. coli* DH10B cells by electroporation as follows: a 30µl aliquot of DH10B electrocompetent cells (Invitrogen) was thawed on ice and 2µl of the BP reaction mix was added. The mixture was transferred to a chilled 0.1 cm electroporation cuvette and the cells
5 electroporated using a BioRad Gene-Pulser™ according to the manufacturer's recommended protocol. SOC media (0.5ml) which had been pre-warmed to room temperature was added immediately after electroporation. The mixture was transferred to a 15 ml snap-cap tube and incubated, with shaking (220 rpm) for 1h at 37°C. Aliquots of the transformation mixture (10µl and 50µl) were then plated on L-broth (LB) plates containing
10 kanamycin (40µg/ml) and incubated overnight at 37°C.

Plasmid mini-prep DNA was prepared from 5ml cultures from 6 of the resultant colonies using a Qiaprep Turbo 9600 robotic system (Qiagen). Plasmid DNA (200-500ng) was subjected to DNA sequencing with 21M13 and M13Rev primers using the BigDyeTerminator system (Applied Biosystems cat. no. 4390246) according to the
15 manufacturer's instructions. The primer sequences are shown in Table 1. Sequencing reactions were purified using Dye-Ex columns (Qiagen) or Montage SEQ 96 cleanup plates (Millipore cat. no. LSKS09624) then analyzed on an Applied Biosystems 3700 sequencer.

Plasmid eluate (2µl) from one of the clones which contained the correct sequence
20 (pENTR-INSP094-6HIS, plasmid ID 13979, figure 11) was then used in a recombination reaction containing 1.5µl of either pEAK12d vector or pDEST12.2 vector (figures 9 & 10) (0.1 µg / µl), 2µl LR buffer and 1.5µl of LR clonase (Invitrogen) in a final volume of 10µl. The mixture was incubated at RT for 1h, stopped by addition of proteinase K (2µg) and incubated at 37°C for a further 10 min. An aliquot of this reaction (1µl) was used to
25 transform *E. coli* DH10B cells by electroporation as follows: a 30µl aliquot of DH10B electrocompetent cells (Invitrogen) was thawed on ice and 1µl of the LR reaction mix was added. The mixture was transferred to a chilled 0.1 cm electroporation cuvette and the cells electroporated using a BioRad Gene-Pulser™ according to the manufacturer's recommended protocol. SOC media (0.5ml) which had been pre-warmed to room
30 temperature was added immediately after electroporation. The mixture was transferred to a 15ml snap-cap tube and incubated, with shaking (220 rpm) for 1h at 37°C. Aliquots of the transformation mixture (10µl and 50µl) were then plated on L-broth (LB) plates containing ampicillin (100µg/ml) and incubated overnight at 37°C.

Plasmid mini-prep DNA was prepared from 5ml cultures from 6 of the resultant colonies subcloned in each vector using a Qiaprep Turbo 9600 robotic system (Qiagen). Plasmid DNA (200-500 ng) in the pEAK12d vector was subjected to DNA sequencing with pEAK12F and pEAK12R primers as described above. Plasmid DNA (200-500ng) in the pDEST12.2 vector was subjected to DNA sequencing with 21M13 and M13Rev primers as described above. Primers sequences are shown in Table 1.

CsCl gradient purified maxi-prep DNA was prepared from a 500ml culture of one of each of the sequence verified clones (pEAK12d-INSP094-6HIS, plasmid ID number 13981, figure 12, and pDEST12.2-INSP094-6HIS, plasmid ID 13980, figure 13) using the method described by Sambrook J. et al., 1989 (in Molecular Cloning, a Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press), Plasmid DNA was resuspended at a concentration of 1µg/µl in sterile water and stored at -20°C.

Example 5: Expression and purification of INSP094

Further experiments may now be performed to determine the tissue distribution and expression levels of the INSP094 polypeptides *in vivo*, on the basis of the nucleotide and amino acid sequence disclosed herein.

The presence of the transcripts for INSP094 may be investigated by PCR of cDNA from different human tissues. The INSP094 transcripts may be present at very low levels in the samples tested. Therefore, extreme care is needed in the design of experiments to establish the presence of a transcript in various human tissues as a small amount of genomic contamination in the RNA preparation will provide a false positive result. Thus, all RNA should be treated with DNase prior to use for reverse transcription. In addition, for each tissue a control reaction may be set up in which reverse transcription was not undertaken (a -ve RT control).

For example, 1µg of total RNA from each tissue may be used to generate cDNA using Multiscript reverse transcriptase (ABI) and random hexamer primers. For each tissue, a control reaction is set up in which all the constituents are added except the reverse transcriptase (-ve RT control). PCR reactions are set up for each tissue on the reverse transcribed RNA samples and the minus RT controls. INSP094-specific primers may readily be designed on the basis of the sequence information provided herein. The presence of a product of the correct molecular weight in the reverse transcribed sample together

with the absence of a product in the minus RT control may be taken as evidence for the presence of a transcript in that tissue. Any suitable cDNA libraries may be used to screen for the INSP094 transcripts, not only those generated as described above.

The tissue distribution pattern of the INSP094 polypeptides will provide further useful
5 information in relation to the function of those polypeptides.

In addition, further experiments may now be performed using the pEAK12d-INSP094-6HIS expression vectors. Transfection of mammalian cell lines with these vectors may enable the high level expression of the INSP094 proteins and thus enable the continued investigation of the functional characteristics of the INSP094 polypeptides. The following
10 material and methods are an example of those suitable in such experiments:

Cell Culture

Human Embryonic Kidney 293 cells expressing the Epstein-Barr virus Nuclear Antigen (HEK293-EBNA, Invitrogen) are maintained in suspension in Ex-cell VPRO serum-free medium (seed stock, maintenance medium, JRH). 16 to 20 hours prior to transfection
15 (Day-1), cells are seeded in 2x T225 flasks (50ml per flask in DMEM / F12 (1:1) containing 2% FBS seeding medium (JRH) at a density of 2×10^5 cells/ml). The next day (transfection day 0) transfection takes place using the JetPEITM reagent (2 μ l/ μ g of plasmid DNA, PolyPlus-transfection). For each flask, plasmid DNA is co-transfected with GFP (fluorescent reporter gene) DNA. The transfection mix is then added to the 2xT225
20 flasks and incubated at 37°C (5%CO₂) for 6 days. Confirmation of positive transfection may be carried out by qualitative fluorescence examination at day 1 and day 6 (Axiovert 10 Zeiss).

On day 6 (harvest day), supernatants from the two flasks are pooled and centrifuged (*e.g.* 4°C, 400g) and placed into a pot bearing a unique identifier. One aliquot (500 μ l) is kept for
25 QC of the 6His-tagged protein (internal bioprocessing QC).

Scale-up batches may be produced by following the protocol called "PEI transfection of suspension cells", referenced BP/PEI/HH/02/04, with PolyEthyleneImine from Polysciences as transfection agent.

Purification process

30 The culture medium sample containing the recombinant protein with a C-terminal 6His tag is diluted with cold buffer A (50mM NaH₂PO₄; 600mM NaCl; 8.7 % (w/v) glycerol, pH

7.5). The sample is filtered then through a sterile filter (Millipore) and kept at 4°C in a sterile square media bottle (Nalgene).

The purification is performed at 4°C on the VISION workstation (Applied Biosystems) connected to an automatic sample loader (Labomatic). The purification procedure is composed of two sequential steps, metal affinity chromatography on a Poros 20 MC (Applied Biosystems) column charged with Ni ions (4.6 x 50 mm, 0.83ml), followed by gel filtration on a Sephadex G-25 medium (Amersham Pharmacia) column (1,0 x 10cm).

For the first chromatography step the metal affinity column is regenerated with 30 column volumes of EDTA solution (100mM EDTA; 1M NaCl; pH 8.0), recharged with Ni ions through washing with 15 column volumes of a 100mM NiSO₄ solution, washed with 10 column volumes of buffer A, followed by 7 column volumes of buffer B (50 mM NaH₂PO₄; 600mM NaCl; 8.7% (w/v) glycerol, 400mM; imidazole, pH 7.5), and finally equilibrated with 15 column volumes of buffer A containing 15mM imidazole. The sample is transferred, by the Labomatic sample loader, into a 200ml sample loop and subsequently charged onto the Ni metal affinity column at a flow rate of 10ml/min. The column is washed with 12 column volumes of buffer A, followed by 28 column volumes of buffer A containing 20mM imidazole. During the 20mM imidazole wash loosely attached contaminating proteins are eluted from the column. The recombinant His-tagged protein is finally eluted with 10 column volumes of buffer B at a flow rate of 2ml/min, and the eluted protein is collected.

For the second chromatography step, the Sephadex G-25 gel-filtration column is regenerated with 2ml of buffer D (1.137M NaCl; 2.7mM KCl; 1.5mM KH₂PO₄; 8mM Na₂HPO₄; pH 7.2), and subsequently equilibrated with 4 column volumes of buffer C (137mM NaCl; 2.7mM KCl; 1.5mM KH₂PO₄; 8mM Na₂HPO₄; 20% (w/v) glycerol; pH 7.4). The peak fraction eluted from the Ni-column is automatically loaded onto the Sephadex G-25 column through the integrated sample loader on the VISION and the protein is eluted with buffer C at a flow rate of 2 ml/min. The fraction was filtered through a sterile centrifugation filter (Millipore), frozen and stored at -80°C. An aliquot of the sample is analyzed on SDS-PAGE (4-12% NuPAGE gel; Novex) Western blot with anti-His antibodies. The NuPAGE gel may be stained in a 0.1 % Coomassie blue R250 staining solution (30% methanol, 10% acetic acid) at room temperature for 1h and subsequently

destained in 20% methanol, 7.5% acetic acid until the background is clear and the protein bands clearly visible.

Following the electrophoresis the proteins are electrotransferred from the gel to a nitrocellulose membrane. The membrane is blocked with 5% milk powder in buffer E (137mM NaCl; 2.7mM KCl; 1.5mM KH_2PO_4 ; 8mM Na_2HPO_4 ; 0.1% Tween 20, pH 7.4) for 1h at room temperature, and subsequently incubated with a mixture of 2 rabbit polyclonal anti-His antibodies (G-18 and H-15, 0.2 $\mu\text{g}/\text{ml}$ each; Santa Cruz) in 2.5% milk powder in buffer E overnight at 4°C. After a further 1 hour incubation at room temperature, the membrane is washed with buffer E (3 x 10min), and then incubated with a secondary HRP-conjugated anti-rabbit antibody (DAKO, HRP 0399) diluted 1/3000 in buffer E containing 2.5% milk powder for 2 hours at room temperature. After washing with buffer E (3 x 10 minutes), the membrane is developed with the ECL kit (Amersham Pharmacia) for 1 min. The membrane is subsequently exposed to a Hyperfilm (Amersham Pharmacia), the film developed and the western blot image visually analysed.

For samples that showed detectable protein bands by Coomassie staining, the protein concentration may be determined using the BCA protein assay kit (Pierce) with bovine serum albumin as standard.

Furthermore, overexpression or knock-down of the expression of the polypeptides in cell lines may be used to determine the effect on transcriptional activation of the host cell genome. Dimerisation partners, co-activators and co-repressors of the INSP094 polypeptide may be identified by immunoprecipitation combined with Western blotting and immunoprecipitation combined with mass spectroscopy.

Example 6: Assays for testing specificity, potency, and efficacy of chemokines

Several assays have been developed for testing specificity, potency, and efficacy of chemokines using cell cultures or animal models, for example *in vitro* chemotaxis assays (Proudfoot A, *et al.* J Biol Chem 276: 10620-10626, 2001; Lusti-Narasimhan M *et al.*, J Biol Chem, 270: 2716-21, 1995), or mouse ear swelling (Garrigue JL *et al.*, Contact Dermatitis, 30: 231-7, 1994). Many other assays and technologies for generating useful tools and products (antibodies, transgenic animals, radiolabeled proteins, *etc.*) have been described in reviews and books dedicated to chemokines (Methods Mol. Biol vol. 138, "Chemokines Protocols", edited by Proudfoot AI *et al.*, Humana Press Inc., 2000; Methods

Enzymol, vol. 287 and 288, Academic Press, 1997), and can be used to verify, in a more precise manner, the biological activities of the chemokine-like polypeptides of the invention and related reagents in connection with possible therapeutic or diagnostic methods and uses.

- 5 The following *in vitro* cell-based tri-replicas assays measure the effects of the protein of the invention on cytokine secretion induced by Concanavalin A (Con A) acting on different human peripheral blood mononuclear cells (hPBMC) cells as measured by a cytokine bead array (CBA) assay for IL-2, IFN- γ , TNF- α , IL-5, IL-4 and IL-10 such as the Human Th1/Th2 Cytokine CBA kit (Becton-Dickinson).
- 10 The optimal conditions are 100 000 cells/well in 96-well plates and 100 μ l final in 2% glycerol. The optimal concentration of mitogen (ConA) is 5ng/ml. The optimal time for the assay is 48h. The read-out choice is the CBA.

1 Purification of Human PBMC from a buffy coat

- The buffy coat 1 to 2 is diluted with DMEM. 25ml of diluted blood was thereafter slowly
15 added onto a 15ml layer of Ficoll in a 50 ml Falcon tube, and tubes are centrifuged (2000 rpm, 20 min, at RT without brake). The interphase (ring) is then collected and the cells are washed with 25 ml of DMEM followed by a centrifuge step (1200 rpm, 5 min). This procedure is repeated three times. A buffy coat gives approximately 600 x 10⁶ total cells.

2 Screening

- 20 80 μ l of 1.25 x 10⁶ cells/ml are diluted in DMEM+2.5% Human Serum+1% L-Glutamine+1% Penicillin-Streptomycin and thereafter added to a 96 well microtiter plate.

10 μ l are added per well (one condition per well): Proteins were diluted in PBS+20%Glycerol (the final dilution of the proteins is 1/10).

- 10 μ l of the ConA Stimulant (50 μ g/ml) are then added per well (one condition per well -
25 the final concentration of ConA is 5 μ g/ml)

After 48 h, cell supernatants are collected and human cytokines are measured by Human Th1/Th2 Cytokine CBA Kit Becton-Dickinson.

3 CBA analysis

(for more details, refer to the manufacturer's instructions in the CBA kit)

- 30 i) Preparation of mixed Human Th1/Th2 Capture Beads

The number of assay tubes that are required for the experiment are determined.

Each capture bead suspension is vigorously vortexed for a few seconds before mixing. For each assay to be analysed, 10µl aliquot of each capture bead are added into a single tube labelled "mixed capture beads". The bead mixture is thoroughly vortexed.

5 ii) Preparation of test samples

Supernatants are diluted (1:4) using the Assay Diluent (20µl of supernatants + 60µl of Assay Diluent). The sample dilution is then mixed before transferring samples into a 96 well conical bottomed microtiter plate (Nunc).

iii) Human Th1/Th2 Cytokine CBA Assay Procedure

- 10 50µl of the diluted supernatants are added into a 96 well conical bottomed microtiter plate (Nunc). 50µl of the mixed capture beads are added followed by 50µl addition of the Human Th1/Th2 PE Detection Reagent. The plate is then incubated for 3 hours at RT and protected from direct exposure to light followed by centrifugation at 1500rpm for 5 minutes. The supernatant is then carefully discarded. In a subsequent step, 200µl of wash
- 15 buffer are twice added to each well, centrifuged at 1500rpm for 5 minutes and supernatant carefully discarded. 130µl of wash buffer are thereafter added to each well to resuspend the bead pellet. The samples are finally analysed on a flow cytometer. The data are then analysed using the CBA Application Software, Activity Base and Microsoft Excel software.
- 20 From the read-out of the assay it can be evaluated whether *in vitro*, the protein of the invention has a consistent inhibitory effect on all cytokines tested (IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10).

Moreover, based on the EC50 value, it can be easily evaluated which cytokine is inhibited the most and then derive the specific auto-immune / inflammatory disease, which is known

25 to be particularly linked to that cytokine.

Table 1 INSP094 cloning and sequencing primers

Primer	Sequence (5'-3')
INSP094-CP1	ATG GCA TTT TCT GCT ACC AA
INSP094-CP2	TGT GCT AGA ATA TT GTT TTA CC
INSP094-EX1	AA GCA GGC TTC <u>GCC ACC</u> ATG GCA TTT TCT GCT ACC AA
INSP094-EX2	<i>GTG ATG GTG ATG GTG</i> GAA TAT TGT TTT ACC CCC TG
GCP Forward	G GGG ACA AGT TTG TAC AAA AAA GCA GGC TTC <u>GCC ACC</u>
GCP Reverse	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT TCA <i>ATG GTG ATG GTG ATG GTG</i>
pEAK12F	GCC AGC TTG GCA CTT GAT GT
pEAK12R	GAT GGA GGT GGA CGT GTC AG
21M13	TGT AAA ACG ACG GCC AGT
M13REV	CAG GAA ACA GCT ATG ACC
T7	TAA TAC GAC TCA CTA TAG G
T3	ATT AAC CCT CAC TAA AGG

5

Underlined sequence = Kozak sequence**Bold** = Stop codon*Italic* sequence = His tag

List of sequences**SEQ ID NO:1 (INSP093 nucleotide sequence exon A)**

5 1 ATGTCAGCAC AACATGGTCT TGTTTCCAAA TTTGGGCTGG GGCTTCTGCT CCTTGGGGAC
 61 AAATACTTTC AAAGACATGA ACAATCAAAA CCTCATCAAG AAGAAATAGA CAACCTGCAT
 121 AGCCCT

SEQ ID NO:2 (INSP093 polypeptide sequence exon A)

10 1 MSAQHGLVSK FGLGLLLLGD KYFORHEQSK PHQEEIDNLH SP

SEQ ID NO:3 (INSP093 nucleotide sequence exon B)

15 1 GACCTGCCCA CGCCGGGACA CCCGGTGACA CTCCACTCCC TCTGCTTTTG CAGCCCCCGG
 61 GGGACCCTCC TCGAGGGGCC CATGTCTTCT GGGTTCCATC GCTTTGAGGT AGAAAATCTG
 121 AGGCCTCAAA CTGCCCCCAA AGCAGGCAAA GGTCAGATGT GTGGAGAGAG GATGGCGAGG
 181 ATGGCAAGGA CGGCCAAGGA GGGTCGCCCC AGGTGCCTGG ACCCAGGTTT GTCCCGCACC
 241 CCGCACCCTG GCCCACATGT CTTCTTCCC CATAGCCCCA CCCCAGCATC CTGGCACCAG
 301 TGGGCTCCTG GTGGCACTGG CTGGATGCTG

SEQ ID NO:4 (INSP093 polypeptide sequence exon B)

25 1 DLPTPGHPVT LHSLCFCSPR GTLLEGPMSS GFHREFEVENL RPQTAPKAGK GQMCGERMAR
 61 MARTAKEGRP RCLDPGLSRT PHPGPHVFLP HSPTPASWHQ WAPGGTGWML

SEQ ID NO:5 (INSP093 partial nucleotide sequence)

30 1 ATGTCAGCAC AACATGGTCT TGTTTCCAAA TTTGGGCTGG GGCTTCTGCT CCTTGGGGAC
 61 AAATACTTTC AAAGACATGA ACAATCAAAA CCTCATCAAG AAGAAATAGA CAACCTGCAT
 121 AGCCCTGACC TGCCACGCC GGGACACCCG GTGACACTCC ACTCCCTCTG CTTTTCAGC
 181 CCCCGGGGGA CCCTCCTCGA GGGCCCCATG TCTTCTGGGT TCCATCGCTT TGAGGTAGAA
 241 AATCTGAGGC CTCAAAGTGC CCCCAGCA GGCAAAGGTC AGATGTGTGG AGAGAGGATG
 301 GCGAGGATGG CAAGGACGGC CAAGGAGGGT CGCCCCAGGT GCCTGGACCC AGGTTTGTCC
 361 CGCACCCCGC ACCCTGGCCC ACATGTCTTC CTTCCCCATA GCCCCACCC AGCATCCTGG
 421 CACCAGTGGG CTCCTGGTGG CACTGGCTGG ATGCTGTAG

SEQ ID NO:6 (INSP093 partial polypeptide sequence)

40 1 MSAQHGLVSK FGLGLLLLGD KYFORHEQSK PHQEEIDNLH SPDLPTGHP VTLHSLCFC
 61 PRGTLLEGPM SSGFHRFEVE NLRPQTAPKA GKGQMCGERM ARMARTAKEG RPRCLDPGLS
 121 RTPHPGPHVF LPHSPTPASW HQWAPGGTGW ML

SEQ ID NO:7 (INSP094 nucleotide sequence exon A)

1 AATACCGAGA ATGATTTTTA TGAGATCTGT GGAAATCAGT CACATCATCA CGACAATGCA
 61 AGAATAAAGA AGTTAGTAGA TGGCCTTGAG TTTTCCCAA CAATGGCATT TTCTGCTACC
 5 121 AAAATAAATA TGTATTTCAG TCAGAACCAC TGGACTATAA GAAGTATATT CCATTCTGGT
 181 TTTTACTGGG GGAAAGGATG TTGCCACAAG ATGTCAGTCC ATTTATTCAT TCATATATCC
 241 AATAGATATT TTATGACCAC TTCCATGTGC CAGGAGATGG CTAAGATCCT TGGGAAGACAG
 301 ATAAAATGCT ACCTACCAAC TCAAAGTCCA GTTAGGGAGT CAGGGGGTAA AACAATATTC

10 SEQ ID NO:8 (INSP094 polypeptide sequence exon A)

1 NTENDFYEIC GNQSHHHDNA RIKKLVDGLE FSQTMAFSAT KINMLFSQNH WTIRSIFHSG
 61 FYWGKGCCHK MSVHLFIHIS NRYFMTTSMC QEMAKILGRQ IKCYLPTQSP VRESGGKTIF

15 SEQ ID NO:9 (INSP094 partial nucleotide sequence)

1 AATACCGAGA ATGATTTTTA TGAGATCTGT GGAAATCAGT CACATCATCA CGACAATGCA
 61 AGAATAAAGA AGTTAGTAGA TGGCCTTGAG TTTTCCCAA CAATGGCATT TTCTGCTACC
 121 AAAATAAATA TGTATTTCAG TCAGAACCAC TGGACTATAA GAAGTATATT CCATTCTGGT
 20 181 TTTTACTGGG GGAAAGGATG TTGCCACAAG ATGTCAGTCC ATTTATTCAT TCATATATCC
 241 AATAGATATT TTATGACCAC TTCCATGTGC CAGGAGATGG CTAAGATCCT TGGGAAGACAG
 301 ATAAAATGCT ACCTACCAAC TCAAAGTCCA GTTAGGGAGT CAGGGGGTAA AACAATATTC

SEQ ID NO:10 (INSP094 partial polypeptide sequence)

25

1 NTENDFYEIC GNQSHHHDNA RIKKLVDGLE FSQTMAFSAT KINMLFSQNH WTIRSIFHSG
 61 FYWGKGCCHK MSVHLFIHIS NRYFMTTSMC QEMAKILGRQ IKCYLPTQSP VRESGGKTIF

SEQ ID NO:11 (INSP094 cloned partial nucleotide sequence)

30

1 ATGGCATTTT CTGCTACCAA AATAAATATG TTATTTCAGTC AGAACCACTG GACTATAAGA
 61 AGTATATTCC ATTCTGGTTT TTTACTGGGGG AAAGGATGTT GCCACAAGAT GTCAGTCCAT
 121 TTATTCATTC ATATATCCAA TAGATATTTT ATGACCACTT CCATGTGCCA GGAGATGGCT
 181 AAGATCCTTG GAAGACAGAT AAAATGCTAC CTACCAACTC AAAGTCCAGT TAGGGAGTCA
 241 GGGGGTAAAA CAATATTCTA GCACA

35

SEQ ID NO:12 (INSP094 cloned partial polypeptide sequence)

1 MAFSATKINM LFSQNHWTIR SIFHSGFYW GKGCCHKMS VHLFIHISN RYFMTTSMCQ
 61 EMAKILGRQI KCYLPTQSPV RESGGKTIF

40